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Protein Crystallography of HIV Reverse Transcriptase for Drug Design

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Beamline(s): X25

Introduction: Human Immunodeficiency Virus (HIV) infection is a life threatening condition for which new treatments are still actively being pursued. The Boehringer Ingelheim drug Viramune® (nevirapine, a non nucleoside Reverse Transcriptase inhibitor, NNRTI) is widely used in highly active antiretroviral therapy (HAART). However, resistant mutations often occur in the NNRTI binding pocket of RT. This is the driving factor for the development of novel NNRTIs with broad antiviral potency, not only against the wild-type virus but also against mutant viruses causing treatment failures. Therefore, drug design still relies heavily on accurate crystal structures of NNRTI complexes with wild-type and mutated RTs.

Methods and Materials: Crystals of RT were grown in presence of various recently synthesized NNRTIs. All data were collected at the NSLS beamline X25 at the maximum flux wavelength (1.1Å). Structures were solved by molecular replacement and refined using CNX (Accelrys) program.

Results: HIV Reverse Transcriptase is a large heterodimer (1000aa) that is recognized as a very challenging protein to crystallize. RT presents a typical polymerase fold with the familiar finger, palm and thumb sub-domains. The flexibility of the thumb and finger sub-domains increases the challenge of obtaining good diffracting crystals. It is known that NNRTI binding modifies the conformation of the thumb domain and reduces its flexibility. This can explain why making an inhibitor complex appears to be the first step toward obtaining good diffracting single crystals of RT. In fact, one of the first high-resolution structures of RT was a complex with nevirapine. The production of large high quality crystals that can generate high-resolution diffraction pattern has been a limiting factor in the field of RT. Most of the time, only small crystals (< 0.1mm) with poor diffraction power are obtained.

In the second cycle of 2002, we have used our access to X25 for a total of 48 hrs in two visits and have collected ten datasets on seven different complexes of RT. The maximum resolution obtained was 2.35Å (Rmerge=7% and I/SigI >2 in last bin). On rotating anode generators, the maximum resolution observed is barely reaching 3Å. In addition, very valuable data have been obtained from 20x20x40um^3 crystals, including a dataset to a resolution of 3.3Å! An average resolution of 2.6Å was obtained with somewhat larger crystals (100-200um). This resolution range might seem low, but with RT, it is excellent.

Conclusions: In our hands, conventional rotating anode generators have failed to produce useful diffraction data on 40um crystals of RT. This particular target requires the high brilliance of synchrotron radiation to collect diffraction data at good resolution (~2.5Å). NSLS X25 has proved to be a well-suited beamline for such research. These data are very useful to guide our drug design effort.